Persistence of supercompensated muscle glycogen in trained subjects after carbohydrate loading

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Goforth, J. R., Harold W., David A. Arnall, Brad L. Bennett, and Patricia G. Law. Persistence of supercompensated muscle glycogen in trained subjects after carbohydrate loading. J. Appl. Physiol. 82(1): 342–347, 1997.—Several carbohydrate (CHO)-loading protocols have been used to achieve muscle glycogen supercompensation and prolong endurance performance. This study assessed the persistence of muscle glycogen supercompensation over the 3 days after the supercompensation protocol. Trained male athletes completed a 6-day CHO-loading protocol that included cycle ergometer exercise and dietary manipulations. The 3-day depletion phase began with 115 min of cycling at 75% peak oxygen uptake followed by 3 × 60-s sprints and included the subjects consuming a low-CHO/high-protein/high-fat (10:41:49%) diet. Subjects cycled 40 min at the same intensity for the next 2 days. During the 3-day repletion phase, subjects rested and consumed a high-CHO/low-protein/low-fat (85:08:07%) diet, including a glucose-polymer beverage. A 3-day postloading phase followed, which involved a moderately high CHO diet (60%) and no exercise. Glycogen values for vastus lateralis biopsies at baseline and postloading days 1–3 were 408 ± 168 (SD), 729 ± 222, 648 ± 186, and 714 ± 196 mmol/kg dry wt, respectively. The CHO-loading protocol increased muscle glycogen by 1.79 times baseline, and muscle glycogen remained near this level during the 3-day postloading period. Results indicate that supercompensated muscle glycogen levels can be maintained for at least 3 days in a resting athlete when a moderate-CHO diet is consumed.

For over two decades, athletes have used carbohydrate (CHO) loading to enhance endurance performance. This practice is based on findings from studies that demonstrated a positive correlation between preexercise muscle glycogen concentration and submaximal exercise duration (1, 3, 16). The classic CHO-loading protocol of Bergström et al. (3) became the predominant method used to elevate muscle glycogen stores above normal levels (i.e., glycogen supercompensation). This 6-day protocol of glycogen depletion and repletion combines exercise with dietary manipulations.

CHO loading is affected by a number of factors (e.g., the degree and method of glycogen depletion and the timing, amount, type, and form of ingested CHO). These factors, in turn, affect the rate and degree of muscle glycogen supercompensation. CHO loading can also produce supercompensation of liver glycogen; however, unlike muscle glycogen, liver glycogen can be converted to free glucose by the enzyme glucose-6-phosphatase (EC 3.1.3.9) and released to the blood (12). Under resting fed conditions, supercompensated liver glycogen will return to normal levels within 2 days (12). Under resting fasting conditions, normal liver glycogen stores become severely depleted after 16–24 h (12).

Unlike the liver, skeletal muscle does not possess glucose-6-phosphatase in significant amounts (29). Therefore, supercompensated skeletal muscle glycogen cannot directly provide blood glucose and must be used within the myocyte. Studies of resting subjects have shown that muscle glycogen levels are not significantly reduced after fasting for 18 h, 24 h, and 3.5 days (20, 22). The mobilization rate of supercompensated muscle glycogen under resting fed conditions, however, has not been studied. After muscle glycogen supercompensation is achieved, it is not known how long supercompensation persists during days of inactivity and moderate-CHO intake.

Maintenance of the elevated muscle glycogen concentrations achieved by a CHO-loading protocol is important for athletes and military personnel (e.g., special operations personnel) who wish to enhance endurance performance. If an athletic event or a military operation is postponed for several days and glycogen levels return to normal, then the anticipated benefits of CHO loading may not be realized. The purpose of this study was to determine whether supercompensated skeletal muscle glycogen levels persist under resting conditions when subjects are consuming a moderate-CHO diet. It was hypothesized that supercompensated muscle glycogen levels achieved by the classic CHO-loading protocol would be maintained for 3 days in trained subjects who abstained from exercise and consumed a moderately high CHO diet.

METHODS

Subjects. Fourteen male athletes (cyclists and runners) participated in this study. All subjects trained regularly and participated in local competitive events. Before giving their written consent to participate, subjects were provided with detailed information on the risks and benefits of all procedures.

Experimental design. The study was approved by the Committee for the Protection of Human Subjects at the Naval Health Research Center (San Diego, CA). All 14 subjects completed a classic CHO-loading protocol combining exhaustive exercise on a cycle ergometer with dietary manipulations. Anthropometric and peak oxygen uptake (VO₂peak)
measurements were made 2 wk before the CHO-loading protocol was started. One week later, a baseline percutaneous muscle biopsy of the vastus lateralis and an overnight fasting blood sample were obtained. These samples were used to determine the subjects' resting muscle glycogen concentrations and blood concentration of free fatty acids, glucose, and lactate before the CHO-loading protocol was begun.

Both dietary composition and exercise mode and intensity are factors that can significantly affect the extent of glycogen supercompensation. Cycling was selected as the mode of exercise for this study because it relies heavily on the vastus lateralis and can produce glycogen depletion with minimal trauma to the muscles (6). The diets and meal plans for the depletion, repletion, and postloading phases were developed by a registered dietitian by using Intake Nutritional Analysis System Version 1.0 (Med-Q, Kensington, MD). Diets were designed for a 79-kg, 178-cm, 31-yr-old man by using the Harris-Benedict equation with an activity factor of 1.5 (Table 1). To increase the uniformity of dietary intake and improve subject compliance, all food and beverage items to be consumed during this study were provided for each subject. A glucose-polymer beverage (Gatorlode, Quaker Oats) was used as a CHO supplement during the glycogen-repletion phase. Subjects drank 245 ml of Gatorlode (~0.70 g CHO/kg body wt) within 20 min after the depletion exercise and an additional 245 ml during the next hour. Additionally, subjects were provided with detailed meal plans and check-off sheets to facilitate documenting food intake. Individual daily compliance to the three diets was calculated as the percentage of the daily prescribed macronutrient actually consumed during each phase (Table 1). The overall subject compliance was reported as the mean of all subjects for all days.

Depletion phase. One week after baseline muscle biopsy and blood draw, the subjects began the 3-day depletion phase. On the 1st day, subjects exercised on a mechanically braked cycle ergometer (model 810, Monark) at 70 revolutions/min (rpm) for 115 min at 75% of their $V_{\text{O}_2\text{peak}}$, followed by three 1-min all-out sprints (~80 rpm at the same resistance) separated by 1-min cycling at 0 W. This modification of the protocol of Costill et al. (5) is intended to deplete the vastus lateralis muscle of glycogen. Days 2 and 3 included 40 min of exercise on the cycle ergometer (i.e., 35 min at 70 rpm at 75% $V_{\text{O}_2\text{peak}}$, followed by three 1-min all-out sprints separated by 1-min cycling at 0 W). During depletion, subjects were provided with a low-CHO-high-protein/high-fat diet (12:41:49%) containing 61 g CHO and 2,109 kcal/day (Table 1) to maintain a low concentration of muscle glycogen (3).

Repletion phase. During the 3-day repletion phase, subjects abstained from physical exercise (e.g., no running, cycling, or other leg exercise) and were relatively inactive. To encourage and monitor compliance, subjects maintained a log of their daily activities and wore a Polar Vantage XL heart rate monitor. The prescribed repletion diet consisted of high-CHO/low-protein/low-fat (83:9:8%) food containing 583 g CHO and 2,797 kcal/day (Table 1). As part of the repletion diet, subjects were provided with three servings (70 g CHO each) of Gatorlode.

Postloading phase. On the 1st day of the postloading phase, all subjects had a second muscle biopsy and blood draw. Subjects were then randomly assigned to either group I ($n = 7$) or group II ($n = 7$). Groups I and II underwent a third muscle biopsy and blood draw on postloading days 2 and 3, respectively. As in the repletion phase, subjects continued to abstain from physical exercise and wore a heart rate monitor. To maintain the elevated level of muscle glycogen during postloading, subjects were provided with meal plans for a moderately high-CHO diet containing 418 g CHO and 2,769 kcal/day (Table 1).

Sampling procedures. During the 2 wk before the depletion phase, subjects reported to the laboratory for anthropometric and $V_{\text{O}_2\text{peak}}$ measurements and for a baseline muscle biopsy and blood draw. $V_{\text{O}_2\text{peak}}$ was determined by using a modification of the protocol of Kasch et al. (17), which included a 4-min low-intensity (~25 W) warm-up period followed by five exercise stages. For stages I, II, and III, subjects pedaled for 3 min at 70 rpm at 20, 40, and 60% of their age-predicted (220 – age) maximum heart rate ($HR_{\text{max}}$), respectively. Stage IV increased to 5 min at 70% $HR_{\text{max}}$. Stage V was an all-out sprint for 1–3 min until volitional fatigue. Respiratory gases were measured at 15-s intervals by using standard open-circuit spirometry.

Muscle biopsies (50–120 mg) were obtained with a Stille biopsy needle (5 mm OD) by using the Bergström percutaneous biopsy method, as modified by Evans et al. (8). Samples used for histochemical analysis were extracted from the biopsy needle, placed in embedding medium (Cryo OCT compound), and frozen in isopentane cooled with liquid nitrogen. The mounted samples were wrapped in aluminum foil and stored at −80°C until analysis. Samples used to determine glycogen concentration were quickly removed from the biopsy needle, placed in a cryotube, immediately frozen in liquid nitrogen, and stored at −80°C until analysis.

Venous blood samples were obtained from the antecubital vein to determine glucose, lactate, and free fatty acid levels. Ten-milliliter blood samples were collected in EDTA-treated
tubes, placed on ice, and then centrifuged. Plasma aliquots were frozen and stored in cryotubes at −80°C until analyzed.

Analytic procedures. Percent body fat was estimated from four-site skinfold thickness and body density (7). Body weight was determined to ± 0.1 kg on an electronic digital scale.

Inspired air volume was measured by using a dry-seal gas meter (model REP 9200, Rayfield Equipment). Mixed expired air was analyzed for oxygen and carbon dioxide concentration by using gas analyzers (models OM-11 and LB-2, respectively, Beckman). The gas analyzers were calibrated before and after each test by using standard gases.

Muscle fiber type was determined by using histochemical properties of myofibrillar adenosine triphosphatase, following the method of Nwoye et al. (25). Fiber type composition and fiber area were estimated from a sample of 175 and 100 fibers, respectively, by using an imaging-processing system (model IP-512, Perceptive Systems) described by Martin et al. (23).

Muscle samples were lyophilized for 10–12 h and prepared for biochemical analysis by using the method of Harris et al. (9). Under a dissection microscope, dried samples were teased apart to remove connective tissue, fat, and blood. Each sample was minced with a scalpel, divided into three subsamples, and hydrolyzed in 1 N HCl. Glycogen concentration of each subsample was determined from glycosyl units and assayed in duplicate by using the fluorometric method of Lowry and Passonneau (21). A reference blank was read after every fifth sample to verify instrument stability. Concentrations of subsamples were calculated from standard curves. The coefficient of variation was calculated from values obtained for the subsamples of each biopsy (n = 124), with a mean of 1.40 ± 0.31 mg dry wt (DW).

Glucose and lactate concentrations were measured in triplicate by using a glucose-lactate analyzer (model 2300, Yellow Springs Instruments). Free fatty acids were determined in triplicate by using the method of Novak (24).

Statistical analyses. Plasma metabolite and muscle glycogen levels measured on days 2 and 3 of the postloading phase were compared initially by using independent sample Student’s t-tests. There were no statistical differences between postloading day 2 (n = 7) and day 3 (n = 7) measurements of glucose, lactate, free fatty acids, and glycogen; therefore, these data were combined (n = 14) for further analysis. Data from postloading days 2 and 3 are presented separately in Figs. 1 and 2. All data were then analyzed by using a repeated-measures multifactor analysis of variance (MANOVA). Repeated-measures one-factor analysis of variance (ANOVA) was then performed for each variable. If overall significant differences were found between sampling times with single-factor ANOVA, protected least significant difference (PLSD) tests were used to make pairwise comparisons. Pearson correlation coefficients were calculated to test for associations between the following: fiber types, CHO intake, glycogen concentrations, and the maximum change in

![Fig. 1. Plasma concentrations of glucose (A), lactate (B), and free fatty acids (C) at each sampling time. Values are means ± SD; n, no. of subjects. Brackets indicate data were combined for analysis. There were no significant differences.](image)
glycogen concentrations after CHO loading. A 95% confidence level was used to detect significant differences in all tests.

RESULTS

The mean age, weight, and height of all subjects were 31 ± 4.2 (SD) yr, 78.6 ± 7.8 kg, and 178.0 ± 4.6 cm, respectively. Mean percent body fat of all subjects was 16 ± 2% (SD). The mean V02peak for all subjects was 54 ± 6.8 (SD) ml·kg⁻¹·min⁻¹. A review of the daily activity logs and heart rate data during the repletion and postloading phases revealed that subjects complied with a relatively inactive lifestyle (e.g., heart rates ranged from 45 to 120 beats/min) as instructed. The subjects were also highly compliant with the dietary meal plans for the 3-day depletion (93.2 ± 4.0%, 3-day repletion (83.6 ± 6.1%), and the 2- or 3-day postloading (81.4 ± 9.6%) diets. Subjects had a mean compliance (i.e., actual vs. prescribed) of 86 ± 6.6% with all meal plans (Table 1). The CHO intake during depletion was 53 ± 9 g, well below the 61 g in the prescribed diet. Also, the mean CHO intake during repletion and postloading was 720 ± 119 and 332 ± 41 g CHO, respectively. Glucose-polymer beverages contributed a mean of 22% (160 g/day) of the dietary CHO during the repletion.

Subjects' mean fiber type distribution was 47 ± 11.5% (SD) type I and 53 ± 11.6% type II, with areas of 4,930 ± 1,110 and 6,020 ± 1,844 µm², respectively. There were no significant correlations between the percentage or area of type I or type II fibers and glycogen levels at any point in the study. There was no significant correlation between fiber type composition, peak muscle glycogen, and grams of CHO consumed during the repletion phase.

The repeated-measures MANOVA revealed an overall significant difference (F = 10.6, P ≤ 0.0001) among metabolite measurements over time. Plasma concentrations of glucose, lactate, and free fatty acids did not vary significantly between sampling times (Fig. 1).

The mean coefficient of variation for glycogen determination among the three subsamples from all biopsies was 3.7%. Mean muscle glycogen concentrations varied by sample time (F = 12.0, P ≤ 0.005). Mean glycogen concentrations on days 1-3 of postloading (729, 648, and 714 mmol/kg DW, respectively) did not differ from each other but were all significantly elevated above baseline (PLSD = 258.7, P = 0.005; Fig. 2). There was a slight but statistically significant negative correlation of baseline glycogen levels with maximum muscle glycogen levels achieved after CHO loading (r² = 0.5, P ≤ 0.008).

DISCUSSION

It is generally accepted that endurance performance during submaximal (i.e., 60–75% maximal oxygen uptake) steady-state exercise is highly correlated with the preexercise muscle glycogen concentration (1, 3). Supercompensated muscle glycogen stores can be achieved by following one of several CHO-loading protocols (13). The most important prerequisites for achieving glycogen supercompensation by the classic method are having the subjects deplete the glycogen in the involved muscle groups and then eat a high-CHO diet (3). The magnitude of muscle glycogen depletion influences the rate of glycogen synthesis (30). The glycogen repletion process is biphasic, exhibiting a rapid early phase (<24 h) and a slow phase lasting for several days. The rate-limiting enzyme for glycogen synthesis is glycogen synthase (EC 2.4.1.11), which exists in both inactive-D and active-I forms. Exercise stimulates the conversion from the inactive D- to the active I-form via hormonal and nonhormonal mechanisms and increases the muscle cell sensitivity to insulin and permeability to glucose. Trained individuals have increased activity of glycogen synthase and higher resting muscle glycogen levels (15). Endurance-trained individuals typically have resting muscle glycogen concentrations of ~530 mmol/kg DW (27). The baseline muscle glycogen values of our subjects (408 ± 168 mmol/kg DW) were lower than those of endurance athletes but approximated those of military special forces personnel (404 ± 126 mmol/kg DW) and other elite military units (14). The similarity of the glycogen values was important because this research was designed to address the requirements of military special operations personnel performing physical endurance tasks (e.g., prolonged swims, cross-country skiing, and overland hikes with heavy loads).

Under some conditions, intensive exhaustive running has proven ineffective as a stimulus for muscle glycogen supercompensation (5). High-intensity and/or exhaustive running (e.g., a marathon) might produce muscle damage, muscle membrane disruption, and inflammation. These effects reduce glucose transport and/or the activation of glycogen synthase (2, 7), interfering with glycogen repletion. Glycogen supercompensation is best achieved when the exercise is largely concentric and the mode of exercise (e.g., cycling) does not disrupt the mechanisms of glycogen synthesis. To avoid potential muscle damage, the present study used a moderate-intensity cycle exercise to achieve glycogen depletion. This follows similar protocols that have achieved significant decreases in glycogen levels and
subsequent supercompensation in the involved muscle groups (16).

To achieve glycogen supercompensation, researchers have recommended using a diet containing 525–650 g CHO/day (8–10.5 g CHO/kg body wt) for 3 days (13). Subjects in the present study ingested a mean of 720 ± 119 g CHO (9.3 ± 1.8 g CHO/kg body wt) daily during the repletion phase. Costill et al. (6) demonstrated that the positive relationship between glycogen repletion rate and CHO consumption approaches an asymptote at ~650 g CHO/day. In the present study, all but three subjects consumed >650 g CHO/day during the 3-day loading period. This may explain the nonsignificant correlation between CHO intake and supercompensated muscle glycogen concentrations.

Both the amount and timing of postexercise CHO intake can significantly affect the rate of glycogen synthesis (13). Ivy (13) demonstrated maximal rates of muscle glycogen synthesis when at least 0.70 g CHO·kg⁻¹·h⁻¹ were ingested immediately (<20 min) after exercise. In the present study, subjects drank 245 ml of a glucose-polymer beverage containing 50 g of CHO (~0.70 g/kg) within 20 min after exercise and an additional 245 ml over the next hour. Clearly, our study followed a protocol designed to achieve maximum glycogen synthesis throughout the repletion phase.

The muscle glycogen data (Fig. 2) demonstrate that the subjects remained supercompensated on postloading days 1, 2, and 3, with mean glycogen levels of 729, 648, and 714 mmol/kg DW that were 1.79, 1.59, and 1.75 times baseline levels, respectively. These increases approximate supercompensated values reported in other CHO-loading studies of trained subjects (3, 29). There was an inverse relationship between the individuals’ supercompensated glycogen concentrations and their baseline glycogen levels. The physiological significance of this finding requires more research.

Maintaining a low level of physical activity and ingesting a moderate amount of CHO (332 ± 41 g/day) during the 3 days of postloading appears to have sustained the persistence of supercompensated glycogen levels. This was as expected, because resting leg muscles rely almost exclusively (80–90%) on fatty acid oxidation for energy (10), and the relatively small amount of CHO used is derived primarily from blood glucose (18). On the basis of current knowledge of muscle glycogen regulation, as well as previous studies on fasting (20, 22), we would also expect supercompensated muscle glycogen to persist under low-CHO or fasting conditions. Additionally, noninvasive studies of in vivo glycogen kinetics in resting human skeletal muscle indicate a net balance between synthesis and degradation (26).

Other studies have reported that normal muscle glycogen levels do not decrease significantly after subjects fast for 1–3.5 days (20, 22). It appears reasonable to conclude that the absence of glucose-6-phosphatase (EC 3.1.3.9) in the muscle cell, and the low level of glycogen phosphorylase activity (EC 2.4.1.1) in resting muscle, allows for the persistence of normal glycogen concentrations (20, 22) and, on the basis of our study, supercompensated glycogen concentrations for at least 3 days.

The nonsignificant changes in the plasma metabolites (glucose, lactate, and free fatty acids) are consistent with data on resting subjects consuming high-CHO diets (3). These plasma metabolite values are also consistent with a low-physical activity profile and, consequently, low muscle glycogenolysis (3).

CHO-loading studies frequently rely on the subjects’ ability to purchase all foods and accurately recall what was eaten during the dietary regimens. In this study, to facilitate recording food intake, subjects were provided with all foods, meal plans, and check-off lists for each meal. Additionally, subjects were provided with glucose-polymer beverages to consume as a CHO supplement during the repletion phase. These beverages were very effective as a supplement because they constituted 22% (161 g/day) of CHO consumed. Glucose-polymer beverages may also have contributed to dietary compliance, because subjects could achieve high-CHO intake (>650 g) with reduced dietary bulk. These procedures resulted in excellent subject compliance to all prescribed diets.

The hypothesis that supercompensated muscle glycogen levels achieved by the classic CHO-loading protocol can be maintained for 3 days in trained subjects who abstain from exercise and consume a moderate-CHO diet is accepted. However, detraining beyond 3 days may be detrimental to performance. Costill et al. (4) showed that competitive collegiate swimmers experienced a 20% decrease in resting muscle glycogen after just 7 days of detraining. Other studies have demonstrated negative cardiovascular and enzymatic changes after 5–12 days of detraining (11, 19). Therefore, additional research is needed to determine the following: 1) the best postloading exercise and dietary regimens, 2) the duration for which high-CHO diets can be maintained without detrimental effects on health and performance, 3) the frequency with which CHO loading can be practiced by endurance athletes, and 4) the benefits of CHO loading to athletes of different fitness levels.

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